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LC method for the analysis of Oxiconazole in pharmaceutical formulations

Julie Milano, Lisoni M. Morsch, Simone Gonçalves Cardoso *

Departamento de Farmácia Industrial, Programa de Pós-Graduação em Ciência e Tecnologia Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, CEP 97.119-900, RS, Brazil

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Abstract

A LC method has been developed for the quantitative determination of Oxiconazole (Ox) in bulk form, lotion and cream pharmaceutical formulations. The method validation yielded good results including the range, linearity, precision, accuracy, recovery, specificity, robustness, limit of quantitation and limit of detection. The LC separation was carried by reversed phase chromatography using a LiChrocart® C_8 column (125 mm × 4.0 mm i.d., 5 μm particle size). The mobile phase was composed of methanol–0.02 M ammonium acetate buffer (85:15 v/v), pumped isocratically at flow rate 1 ml min^{-1}. The detection was carried out on UV detector at 254 nm. The calibration curve for Ox was linear from 40.0–140.0 μg ml^{-1} range. The precision of this method, calculated as the relative standard deviation (R.S.D.) was 1.57% for lotion and 0.71% for cream. The R.S.D. values for intra- and inter-day precision studies were 0.57 and 1.34%, respectively. The recovery of the drug ranged between 98.84–102.2% (lotion) and 100.54–101.59% (cream). The stability indicating capability of the assays was proved using forced degradation. Chromatograms showed Ox well resolved from the degradation product. © 2002 Published by Elsevier Science B.V.

Keywords: Oxiconazole determination; HPLC of Oxiconazole; Imidazole analysis

1. Introduction

Oxiconazole—2′,4′-dichloro-2-(imidazol-1-yl) acetophenone *O*-(2,4-dichlorobenzyl) oxime nitrate (Fig. 1)—is a synthetic imidazole derivative with a broad-spectrum antifungal activity and is indicated for topical treatment of cutaneous fungal infections. This drug blocks the synthesis of ergosterol, an essential component of the fungal cell membrane, by binding to cytochrome P-450 [1]. Results of in vitro and in vivo studies have indicated that Oxiconazole (Ox) has a broad-spectrum of activity against infections caused by dermatophytes, yeast like fungi, molds and mixed infections due to fungi and gram-positive bacteria [2,3]. Ox is currently available in Brazil in cream and lotion formulations. This drug is not official in any pharmacopoeia yet. The literature revealed no LC methods for the determination of Ox in pharmaceutical formulations. In plasma, Ox was analyzed by gas–liquid chromatographic [4]. The

* Corresponding author. Fax: +55-55-220-8248
E-mail address: simonegc@ccs.ufsm.br (S.G. Cardoso).

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thermodynamic behavior of a series of six imidazole derivatives was investigated over a wide range of column temperatures and complexation by β-cyclodextrin [5]. The aim of this work was to develop and validate in compliance with ICH guideline [6] and USP Pharmacopoeia [7] a LC method for quantitative analysis of Ox in raw material, lotion and cream. As there is no interference between the drug and its decomposition products, this procedure can be used in stability studies.

2. Experimental

2.1. Chemical and reagents

Ox nitrate reference substance was kindly supplied by Roche Laboratories and was certified to contain 100.4%. The drug was used without further purification. Pharmaceuticals containing Ox were obtained commercially and were claimed to contain 10 mg of the drug (as base) per milliliter or per gram of lotion and cream, respectively. Methanol was HPLC grade (Merck, Germany or Tedia, USA). Water was glass-distilled.

2.2. Apparatus and chromatographic conditions

The method developed was performed with a LC system consisting of a Shimadzu SLC-10 A-VP system equipped with a Shimadzu model LC-10 AD-VP pump, a Shimadzu SPD-10 A-VP variable-wavelength UV–Vis detector, a Shimadzu SCL-10A system controller and a Rheodyne injection valve with a 20 μl loop. The detector was set at 254 nm (1.0 a.u.f.s.) and peak areas were integrated automatically by computer using a Class VP® software program (Shimadzu, Kyoto, Japan). Separation was carried out at ambient temperature using a LiChrocart® C_8 (125 mm × 4.0 mm i.d., 5 μm particle size; Merck) column. A column LiChrocart® 4-4 (Merck) guard cartridge system was used to safeguard the analytical column. The mobile phase consisted of methanol–0.02 M ammonium acetate buffer (85:15 v/v), at a flow rate of 1 ml min^{-1}. All calculations concerning the quantitative analysis was performed with external standardization by measurement of peak areas.

2.3. Reference substance solution and samples solutions

2.3.1. Reference substance solution

A quantity of Ox nitrate reference substance equivalent to 20 mg de Ox was accurately weighed and transferred to 50-ml volumetric flask. Methanol was added to make up the volume in order to give a final concentration of 400 μg ml^{-1}. Aliquots of the 5 ml of this solution were transferred into 20-ml volumetric flask and mobile phase added to make up the volume in order to give a final concentration of 100 μg ml^{-1}.

2.3.2. Lotion

A quantity of the lotion containing 50.0 mg of Ox was transferred to a 50-ml volumetric flask and methanol added to make up the volume. Five milliliters of this solution was transferred to a 50-ml volumetric flask and mobile phase added to make up the volume in order to give a final concentration of 100 μg ml^{-1}.

2.3.3. Cream

A quantity of the cream containing 50.0 mg of Ox was transferred to 100-ml volumetric flask with 50 ml of a mixture of chloroform and methanol (1:1) and shaken for 30 min, followed by adding methanol to make up the volume. This sample solution had a concentration of 500 μg ml^{-1}. After centrifugation (15 min at 4000 r.p.m at ±4 °C) and filtration, dilutions were

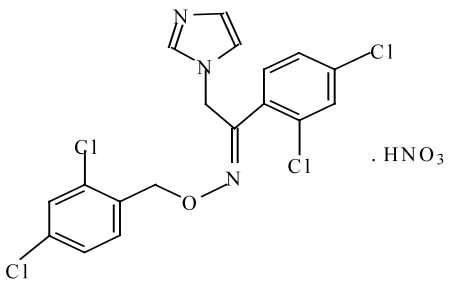

Fig. 1. Chemical structure of Ox nitrate.

made with methanol to give a final concentration of 100 μg ml^{-1}.

2.4. Validation of the method

2.4.1. Linearity

A methanolic stock solution of 400 μg ml^{-1} Ox nitrate reference substance was prepared. Appropriate amounts of these stock solutions were diluted with phase mobile yielding concentrations of 40.0, 60.0, 80.0, 100.0, 120.0 and 140.0 μg ml^{-1}. Triplicate injections were made for each concentration. The linearity of the calibration curves was determined for intra- and inter-day precision in 3 different days. The calibration curves were constructed by plotting the peak area of Ox (y) against concentration of Ox (x), using linear regression analysis.

2.4.2. Precision

The precision of analytical procedure was evaluated through the repeatability of the method by assaying six samples of lotion and 12 samples of cream, at same concentration, during the same day, under the same experimental conditions. The intermediate precision of the method was also evaluated through the performance of the method by another analyst, using a second LC instrument in the same laboratory.

2.4.3. Accuracy

The recoveries were determined at four concentration levels, by adding known amounts of reference substance in the beginning of the process.

2.4.3.1. Lotion. Aliquots (8 ml) of the Ox lotion were transferred volumetrically into 100-ml volumetric flasks, followed by making up to volume with methanol to give a stock solution with concentration of 800 μg ml^{-1}. Portions of 10.0 ml of this stock solution were transferred to 100-ml volumetric flasks where 6.0, 7.0, 8.0 and 9.0 ml of Ox reference solution (400 μg ml^{-1}), equivalent to 24.0, 28.0, 32.0 and 36.0 μg ml^{-1} of Ox, were added. Mobile phase was added to make up the volume in order to give a final concentrations of 104.0; 108.0; 112.0 and 116.0%, respectively, of the sample concentrations used in the assay.

2.4.3.2. Cream. Amounts of cream, equivalent to 40 mg de Ox, were accurately weighed and placed in four 100-ml volumetric flasks. To each was added 5.0, 10.0, 20.0 and 30.0 ml of Ox reference solution (200 μg ml^{-1}), equivalent to 2.5, 5.0, 10.0 and 15.0 μg ml^{-1} of Ox. This sample solutions were processed the same form described in Section 2.3.3. After this procedure, dilutions were made with methanol to give final concentrations of 102.5, 105.0, 110.0 and 115%, respectively, of the sample concentrations used in the assay.

2.4.4. Specificity

Forced degradation studies were performed to evaluate the specificity of the method. Ten milliliters of Ox stock solution (1 mg ml^{-1}) in methanol was taken in three separated 50-ml volumetric flasks and 1.0 M hydrochloric acid, 0.1 M sodium hydroxide and 30% hydrogen peroxide were added to make up the volume. The acid and basic solutions were refluxed at 100 °C for 4 h each. For the oxidative condition, the sample was stored in dark at ambient temperature for 10 h. After the degradation treatments were complete, all the solutions were allowed to cool and neutralized with base or acid (if needed) and diluted with methanol to obtain a concentration of 40 μg ml^{-1}.

2.4.5. Limit of quantitation and limit of detection

The limit of quantitation (LOQ) (taken as the lowest concentration of analyte in a sample, which can be determined with acceptable precision and accuracy) and the limit of detection (LOD) (taken as the lowest absolute concentration of analyte in a sample, which can be detected but not necessarily quantified) were calculated based on the ICH [6].

3. Results and discussion

3.1. Method development

The choice of the method depends on factors such as the nature of the drug, the complexity of the sample and the intended use. In this study the conditions were influenced by the physical–chemi-

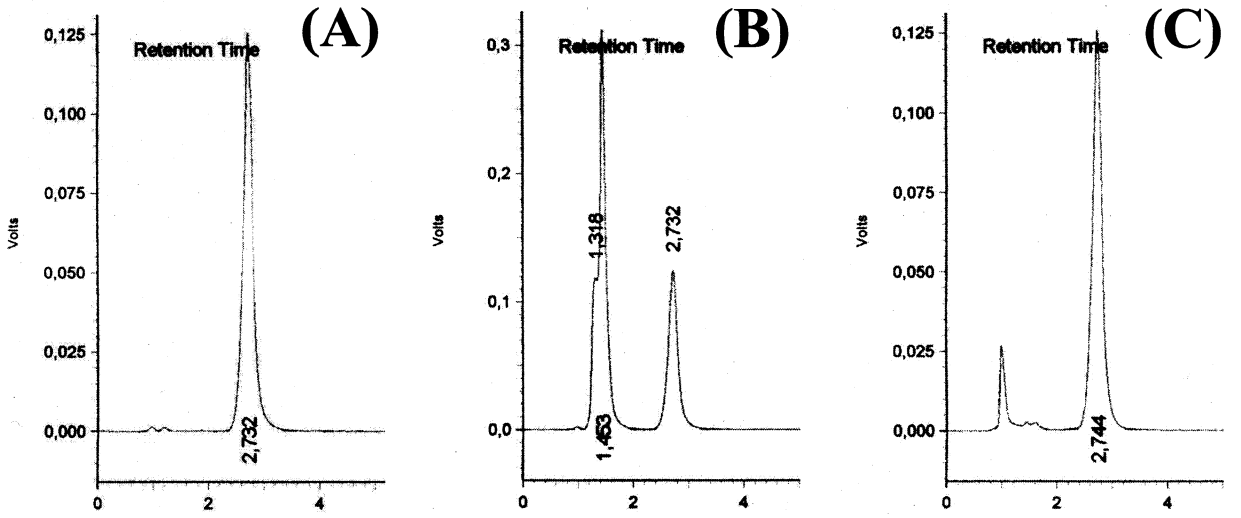

Fig. 2. Chromatograms of Ox, at 100 μg ml^{-1}, (A) reference substance, (B) lotion and (C) cream. Retention time: 2.7 min.

cal properties of Ox such as solubility, polarity, UV absorption and interference. Many commercial pharmaceutical formulations contain benzyl alcohol as a bacteriostatic preservative, which may potentially interfere with analytical methodologies [8]. The most important target was, therefore, to exclude an interference between Ox and benzyl alcohol present in the lotion. The optimum mobile phase was composed by methanol–0.02 M ammonium acetate buffer (85:15 v/v). The flow rate used was 1.0 ml min^{-1}. With this mobile phase, best results were obtained in terms of shape of peak, selectivity and retention time for both pharmaceutical formulations. The retention time for Ox was 2.7 min. No interference from the sample solvent, impurities and dosage form excipients were observed at the detection wavelength (254 nm). Chromatograms of pure Ox, lotion and cream are shown in Fig. 2A–C, respectively.

3.2. Validation of the method

3.2.1. Linearity/range

Linearity parameters of the curve for the Ox were constructed by plotting concentration versus peak area and showed good linearity in the 40–140 μg ml^{-1} ranges. A linear simple regression by the least squares method was applied. The representative linear equation was $y = 18\,979.71 + 14\,893.51x$ ($n = 6$, $r = 0.99996$). At the range, the percent relative standard deviation (R.S.D.) based on the peak area ratios of three replicate injections were found to be between 0.15 and 0.95% intra-day. The inter-day precision was evaluated by comparing the linear regressions of the three standard plots prepared on 3 different days, during a month period, and showed R.S.D. between 0.56 and 1.75%. The analysis of variance of the data indicated no significant difference in slopes of the three calibration curves ($P < 0.01$).

3.2.2. Precision

The repeatability of the method was calculated as the R.S.D. of assays containing Ox in the same range of concentration. The R.S.D. was 1.57 and 0.71% for lotion and cream, respectively, as shown in Table 1. The average purity (%) for intermediate precision was 99.98% (R.S.D. = 0.68%) and 100.14% (R.S.D. = 0.34%) for lotion and cream, respectively.

3.2.3. Accuracy/recovery studies

The accuracy of the LC method was determined by fortifying lotion and cream with known amounts of the Ox reference substance. Mean recoveries for commercial lotion and cream were found to be 101.27 and 101.17%, respectively, as shown in Table 2.

Table 1
Data obtained from commercial sample analyses by HPLC

Product	Theoretical amount (mg[a])	Experimental amount (mg ± S.E.)	Purity (%)	R.S.D. (%)
Lotion ($n = 6$)	10	10.09 ± 0.065	100.99	1.57
Cream ($n = 12$)	10	9.96 ± 0.020	99.62	0.71

S.E., standard error; R.S.D., relative standard deviation.
[a] Units: mg per ml, mg per g of cream.

3.2.4. Specificity

Acid, base and oxidative degradations were performed. For acid condition, 7.2% degradation Ox was obtained after refluxed at 100 °C for 1 h, while 13.8% degradation was obtained after 4 h and showed two additional peaks at 1.2 and 1.6 min. In basic conditions, 52 and 63% of degradation were obtained after 1 and 4 h, respectively. Two small additional peaks were generated at 1.2 and 1.6 min. There was no apparent degradation of the drug with 30% hydrogen peroxide solution at ambient temperature for 10 h.

3.2.5. Robustness/ruggedness

In the order to estimate the robustness, the following parameters were evaluated: stability of analytical solution, length different column and mobile phase. The stability of the reference substance and sample solutions were checked by analyzing these methanolic solutions aged at ± 4 °C in the dark against samples freshly prepared. The results demonstrated that the working reference solution, as well as the sample solutions were stable for up to 22 days. The Ox area response for the assay reference and samples over 22 days did not change. A column packed with the same stationary phase but with different length (LiChocart® C_8 column 250 mm × 4.0 mm i.d.) was investigated at a flow rate 1.2 ml min^{-1}. The retention time for Ox was 4.3 min. The Ox peak areas in both reference and samples decreased. However, the ratio between sample responses and the reference substance response did not change. Analyses of the results of this study showed no significant column-to-column variability. Nevertheless, changing the mobile phase composition from methanol–0.02 M ammonium acetate buffer (85:15 v/v) to methanol–water (75:25 v/v) affected the results of the method. This condition showed a increase in the retention time and bad efficiency of peak, which resulted in an inadequate integration. The same results were obtained when acetic acid 10% was added in mobile phase.

3.2.6. Limit of quantitation and limit of detection

Several approaches are given in the ICH guideline to determine the quantitation and detection limits: visual evaluation, signal-to-noise and standard deviation of the response and the slope. In our study the LOQ and LOD were based on the standard deviation of the response and the slope. LOQ and LOD achieved were 3.75 and 1.2 μg ml^{-1}, respectively.

4. Conclusion

The proposed LC method is simple, rapid and selective for determination of Ox in raw material, lotion and cream, with good linearity, accuracy

Table 2
Recovery of Ox from samples with knows concentrations

Product	Amount of standard (μg ml^{-1})		Recovery (%)[a]
	Spiked	Found	
Lotion	24.00	24.53	102.20
	28.00	28.59	102.10
	32.00	31.63	98.84
	36.00	36.70	101.94
Cream	2.5	2.53	101.22
	5.0	5.08	101.59
	10.0	10.13	101.34
	15.0	15.08	100.54

[a] Mean of three replicate analysis.

and precision. The suggested technique can be used in quality control of formulations containing Ox. Since the results of spiking experiments and forced degradation showed no interference with the Ox peak, the method is both specific and stability-indicating.

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